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Section 5 - Vol I of V

Additional Data Applicable To

PP NOS 6F1773, 6F1832, and 7F1898

Toxicology Data

R# 962

June 10, 1977

Report No. 56654/8/77

Diflubenzuron: Analysis of Metabolites
Connected With Methaemoglobinemia

Test Compound:	Dimilin (Diflubenzuron)
Test Specie:	Male Wistar Rats
Number of Animals:	45 rats, 15 per test group, one control group
Route of Administration:	Dietary
Dose:	7.8 grams/kg - mean daily dose - for four days
Testing Laboratory:	Philips - Duphar, B.V. Weesp, The Netherlands

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Methodology: The evaluation of the chemical analytical methods used to analyze for 4-chloroaniline, 4,4'dichloro azobenzene and 4,4'dichloro-azoxybenzene are within the competancy and jurisdiction of Chemistry Branch.

Results: The plasma levels of 4-diloroaniline in rats averaged about 30 ng/ml while levels at 4-chloroaniline in red blood cells were 30 µg/gram of cells explaining the mechanism for the formation at methaemoglobinemia.

Validation: Core - guideline

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Section 6 - Vol 1 of V

Additional Data Applicable to

PP NOS 6F1773, 6F1832 and 7F1898

R # 962

June 10, 1977

Report Number 56645/2/77

The Methaemoglobin and Sulphaemoglobin Forming Properties of DU112307 In Male Rabbits After Prolonged Dietary and Dermal Administration

Experiment 1

Test Compound:	DU 112307 (technical; Batch # 405093
Test Specie:	Male rabbits
Number of Animals:	15 treated, 15 controls
Route of Administration:	Diet
Dose:	640 ppm for 21 days

Experiment 2

A.	Test Compound:	DU 112307 (analytically pure) Batch # 601141
	Test Specie:	Male rabbits

Experiment 2 A. Cont'd.

	Number of Animals:	15 treated, 15 controls
	Dose:	640 ppm for 18 days
	Route:	Dietary
B.	Test Compound:	DU 112307 (technical)
	Test Specie:	Male rabbits
	Number of Animals:	Two groups of 15 treated rabbits each. One control group treated with vehicle only.
	Route:	Dermally
	Dose:	70% test material concentration, 1.5 ml/kg for 18 days. (One of the two groups with Formo-Cibazol 1000 ppm coccidiostat and the other without the coccidiostat).

Methodology - Experiment 1

A group of 15 male rabbits was administered DU 112307 (technical) at dose level of 640 ppm for 21 days. A control group of 15 rabbits received only basal diet. Methemoglobin levels and sulphemoglobin levels were examined in blood samples at 20, 17 days and one hour before treatment; and at five and 24 hours and at 4, 9, 14 and 21 days after treatment.

Methodology - Experiment 2

A. A group of 15 male rabbits was administered DU 112307 (analytically pure) in the diet at 640 ppm for 18 days. A control group received just diet.

B. Two groups of 15 male rabbits were treated dermally with technical DU 112307 at 70%, 1.5 ml/kg for 18 days. One of these groups was given the coccidiostat Formo-Cibazol at a dose of 1000 ppm before and during treatment and recovery. A control group was dermally treated with vehicle and received no coccidiostat.

After the treatment period, there was a recovery period of 21 days for the group fed test material and 4 days for those dermally treated. Methemoglobin values were measured in blood at 31 and one day before treatment and on days 1, 4, 11, 18 after treatment for the orally dosed group and on days 1, 2, 4, 7, 14 and 21 days after treatment and day one and four of the recovery period for animals dermally treated.

Results: Experiment 1.

The report states, "According to the t-Test of Student at days 4, 9, 14 and 21 the methemoglobin levels and after five hours and at 9, and 14 days the 'SulphHb' levels of the compound treated groups were significantly although marginally increased."

Comment: Sulphemoglobin is not a normal constituent of blood. Clarification is needed regarding the presence of sulphemoglobin values in control animals.

Results: Experiment 2. Dermal Testing.

In group 2 (with coccidiostat) there is an increasing trend with time in metHb levels from day 4 of treatment to day 18 with day 11 being

statistically significant over its corresponding control values. Control values from day 4 to 18 also show an increasing trend. Group 3 (without coccidiostat) again shows an increasing trend with again statistical significance evident at day 11.

Results: Experiment 2. Dietary Testing

According to the T test, the group 5 (640 ppm) was significantly increased over controls on days 11 and 18 of treatment and on days 1, 2, 4 and 7 of recovery.

Conclusion: It can be concluded that DU 112307 is a methemoglobin-forming agent in rabbits either orally or dermally when administered at the above stated dose levels.

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Section 7 - Vol. I of V

Additional Data Applicable to
PP NOS. 6F1773, 6F1832, and 7F1898

R # 962

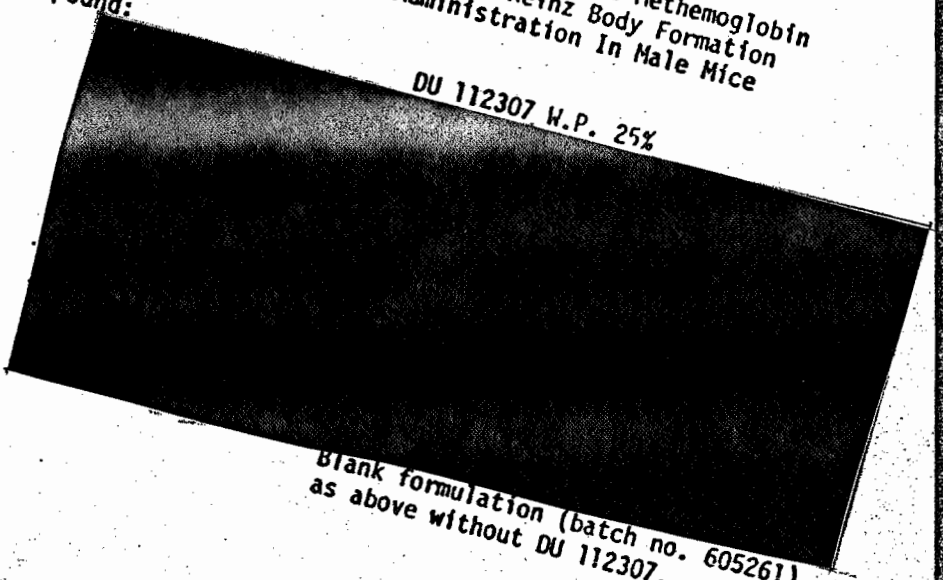
June 10, 1977

Report No. 56645/12/77

The Effect of DU 112307 W.P. 25% On The Methemoglobin And Sulphemoglobin Levels and Heinz Body Formation After A Single Oral Administration In Male Mice

Test Compound:

DU 112307 W.P. 25%



Blank formulation (batch no. 605261) as above without DU 112307.

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Test Specie:	Male Swiss Mice
Number of Animals:	30 mice, 15/test group
Route of Administration:	Gastric intubation
Dose:	10,000 mg/kg
Testing Laboratory:	Philips-Duphar B. V. Weesp, The Netherlands

Methodology: A group of 15 male mice were administered a one percent tragacanth suspension of test formulation at a single dose of 10,000 mg/kg body weight. Animals were housed in stainless steel cages, five per cage. Animals were weighed before dosing and 24 hours post treatment. A group of 15 male mice were treated the same but administered 7,500 mg/kg of blank formulation. Blood samples were taken at 4 hours and 24 hours after treatment. Methemoglobin and sulphemoglobin values and Heinz Bodies were sought.

Results: There were no effects seen in bodyweight after 24 hours. No Heinz bodies could be detected at the two sampling intervals, 4 and 24 hours.

At 4 hours, there was a statistically significant increase in methemoglobinemia in the treated group, as compared to the 4 hour control group.

No appreciable increase was noted for sulphemoglobin at 4 hours or at 24 hours as compared to controls.

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Comment: Sulphemoglobin is not a normal constituent of blood. The existence of sulphemoglobin in mouse blood in control animals found at 4 hours and at 24 hours requires classification.

Validation: Core - Guidelines tentative to clarification under comment.

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Section 8 - Vol. I of V

Additional Data Applicable to

PP NOS. 6F1773, 6F1832, and 7F1898

R # 962

June 10, 1977

Report # 56645/13/77

The Effect Of Du 112307 W.P. 25% On The Methaemoglobin And Sulphaemoglobin Levels And Heinz Body Formation After A Single Dermal Application In Rabbits

Test Compound:	DU 112307 W.P. 25% (Batch No. Fl. 44/703111)
Test Specie:	Male New Zealand White Rabbits
Number of Animals:	30 rabbits, 15 per test group, two test groups
Route of Administration:	Dermal
Dose:	0 - controls and 4,640 mg/kg test group
Test Laboratory:	Philips-Duphar B. V. Weesp, The Netherlands

Methodology: Animals were weighed, housed in stainless steel cages. Room temperature was 18-29°C, relative humidity 70-80%. The day prior to testing, the animals were clipped free of hair around the trunk. Each animal was wrapped in sticking plaster covered with aluminum foil. Test material was introduced between skin and plaster using blunt needle. After 24 hours, coverings were removed and skin was washed with soap and water then dried. Blood was drawn from ear vein at 4 and 24 hours after application to measure methaemoglobin, sulphaemoglobin levels and to stain for Heinz bodies.

Results: No methaemoglobin or sulphaemoglobin levels were detected. No Heinz bodies were found on the prepared slides.

Conclusion: DU 112307 25% W.P. when applied to New Zealand White rabbits at 4,640 mg/kg dermally does not produce methemoglobin or sulphaemoglobin or Heinz bodies according to the methods employed for detection.

Validation: Core - Guidelines

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Section 9 Vol. I of V

Additional Data Applicable to PP NOS. 6F1773,

6F1832, and 7F1898

P # 962

June 10, 1977

Report No. 56645/15/77

The Methemoglobin and Sulphemoglobin and Heinz Body Forming Properties of DU 112307 After Oral Administration To Male Rats During 8 Days

Test Compound:	DU 112307 (technical) in one percent tragacanth vehicle
Test Specie:	Male rats, Wistar Strain
Number of Animals:	30 rats, 15 per test group
Route of Administration:	Oral intubation
Dose:	5,000 mg/kg per day for eight days

Methodology: One group of 15 rats were administered DU 112307 (technical) at 5,000 mg/kg by gastric intubation daily for eight days in a volume of 15 ml/kg bodyweight. Blood samples were taken two days before administration of test material and

at 4 and 24 hours after the first dosing and at 2, 3, 4 and 8 days after start of the repeated dosing and at day 3, 4 hours after dosing that day. Heinz bodies were sought in blood samples taken 4 hours, 24 hours and 8 days after initial treatment.

Results: No Heinz bodies were observed in any of the above sample periods. At one day the methemoglobin values and at day 2, 3, 4, 8 the methemoglobin and sulphemoglobin values were increased in respect to control values.

Conclusion: When DU 112307 (technical) is administered at 5,000 mg/kg p.o. to male Wistar rats, methemoglobin and sulphemoglobin is detected in blood levels statistically significant over control values.

Validation: Core - Guidelines

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Section 10 Vol. I of V

Additional Data Applicable to

PP NOS. 6F1773, 6F1832 and 7F1898

R # 962

June 10, 1977

Report No. 56645/4/77

Acute Oral Toxicity Study With DU 112307 (technical) in Mice

This report submits the laboratory test data information needed to validate report no. 56645/14/73 previously submitted in 1976 without experimental test data and therefore marked invalid.

This information in this report no. 56645/4/77 now validates report no. 56645/14/73 in regards to the results in mice.

Test Laboratory: Philips Duphar, B.V.
Weesp, The Netherlands

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Section 11 Vol. I of V

Additional Data Applicable to

PP NOS. 6F1773, 6F1832 and 7F1898

R # 962

June 10, 1977

Report No. 56645/3/77

Acute Oral Toxicity Study With DU 112307 W.P. 25%
In Mice and Rats

This report submits the laboratory test data information needed to validate report no. 56645/15/73 previously submitted in 1976 without experimental test data and therefore marked invalid.

The information in this report no. 56645/3/77 now validates report no. 56645/14/77.

Test Laboratory: Philips-Duphar B.V.
Weesp, The Netherlands

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Section 12 Vol. I of V

Additional Data Applicable to

PP NOS. 6F1773, 6F1832, and 7F1898

R # 962

June 10, 1977

Report # 56645/2/77

Acute Toxicity In Rats of DU 112307 (Technical)
After Dermal Application

Test Material:	DU 112307 (technical) Batch # 405093
Test Specie:	Male and female rats - SPF Wistar
Number of Animals:	3 groups of 5 males and 5 females
Route of Administration:	Dermal
Doses:	4,640 mg/kg and 10,000 mg/kg
Testing Laboratory:	Philips Duphar, B.V. Weesp, The Netherlands

Methodology: Three groups of SPF Wistar rats, 5 males and 5 females were placed on test. The rats were shaved with clippers around the flanks and back. The clipped trunks were

covered with a strip of plaster. The rats were not abraded. One group received DU 112307 at 10,000 mg/kg in a volume of 20 ml/kg dermally while a second group received 4,640 mg/kg of test material also dermally. The third group received only vehicle-1% tragacanth solution. Blood was taken from the orbital vein before test material administration and at 5 and 24 hours after test material administration. The animals were observed for a subsequent period of two weeks after initiation of test for signs of toxicity.

Results: The LD_{50} is greater than 10,000 mg/kg of bodyweight of DU 112307 (technical) and no increase in methemoglobin or sulphemoglobin were detected under these conditions in rats, during test material administration or during the subsequent two week period.

Validation: Core - Guidelines

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Section 13 Vol 11 of V

Additional Data Applicable to PP Nos. 6F1773, 6F1832 and 7F1898

R# 962

June 10, 1977

Acute Dermal Toxicity Study with DU 112307 (Technical) in Rats

Test Material: DU 112307 Technical

Batch No. 405093

Test Specie: Male and female Wistar rats

Number of Animals: 5 per sex per dose

Route of administration: Dermal

Doses: 4,640 and 10,000 mg/kg

Testing Laboratory: Philips Duphar, B.V.

Weesp, The Netherlands

Methodology: As in report no. 56645/2/77-Section 12-Vol 1 of V

Results: The LD_{50} is greater than 10,000 mg/kg when administered dermally to Wistar rats as a single application of test material suspended in a 1% tragacanth solution.

Validation: Core - Guidelines

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Section 14 Vol 11 of V

Additional Data Applicable to PP Nos. 6F1773, 6F1832 and 7F1898

R# 962

June 10, 1977

Report No. 56645/6/77

Acute Dermal Toxicity Study with DU 112307 W.P. 25% In Rabbits

This report submits the laboratory test data information needed to validate report #56645/17/73 previously submitted in 1976 without experimental test data and therefore marked invalid.

The information in this report #56645/6/77 now validates report #56645/17/73.

Test Laboratory: Philips Duphar, B.V.
Weesp, The Netherlands

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Section 15 Vol 11 of V

Additional Data Applicable to PP Nos. 6F1773, 6F1838 and 7F1898

R# 962

June 10, 1977

Report Number 56645/5/77

Acute Intra-Peritoneal Toxicity Study With DU 112307 (Technical) In Mice

This report submits the laboratory test data information needed to validate report #56645/1/74 previously submitted in 1976 without experimental test data and therefore marked invalid.

The information in this report #56645/5/77 now validates report #56645/1/74.

Test Laboratory: Philips Duphar, B.V.
Weesp, The Netherlands

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Section 16 Vol 11 of V

Additional Data Applicable to PP Nos. 6F1773, 6F1832 and 7F1898

R# 962

June 10, 1977

Tumorigencity of DU 112307 To Mice-Dietary Administration For 80 Weeks

(Reevaluated Pathology Data)

Addendum to Report PDR 170/75685

Results: The individual animal pathology data submitted on two mice does not change the results of the report PDR 170/75685 submitted in 1976.

Test Laboratory: Huntingdon Research Center
Huntingdon, England

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Section 17 of Vol 11 of V

Additional Data Applicable to PP Nos. 6F1773, 6F1832 and 7F1898

R# 962

June 10, 1977

Effects of DU 112307 in Dietary Administration to Rats for 104 Weeks

(Reevaluated Pathology Data)

Results: The individual animal pathology data submitted in this report does not change the results of the report PDR 171/75945 submitted in 1976.

Test Laboratory: Huntingdon Research Center
Huntingdon, England

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Section 18 Vol 11 of V

Additional Data Applicable to PP Nos 6F1773, 6F1832 and 7F1898

R# 962

Submitted June 10, 1977

Report

Macroscopic and Microscopic Examination of Tissues of Sheep and Swine Administered

Diflubenzuron

Laboratory #7E-5790

Diflubenzuron

Pathology Report

Test Compound: Diflubenzuron

Test Species: Swine and Sheep

Number of Swine: 9 female, 2 male controls

6 female, 2 males treated

Number of Sheep: 10 female, 2 male controls

6 females, 3 males treated

Route of Administration: Dietary

Doses: Diflubenzuron - 100 ppm

No duration of time given

Testing Laboratory: Cannon Laboratories, Inc.

Sponsor: Thompson - Hayward Chemical Company

Results: All 24 tissues examined both in control and treated animals showed no macroscopic or microscopic evidence of toxicity attributable to the administration of test material.

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Section 19 - Vol. II of V

Effects of Feeding Dimilin (TH-6040)

Upon Reproduction and Residues In

Sheep and Swine

Progress Report, February 1977

Test Compound: Dimilin (TH-6040)

Test Specie: Columbia-Rambouilet ewes + rams, Dorsets and Hampshire

Number of Sheep: 51 ewes, 6 rams

Number of Swine: 37 gilts, 4 boars

Route of Administration: Dietary

Doses: 100 ppm

Testing Labortors: Department of Animal Science

University of Maryland

College Park, Maryland 20742

Sponsor: Thompson - Hayward Chemical Co.

Methodology: Sheep Project

Fifty - one Columbia - Rambouilet ewes and six ram (two Dorsets and four Hampshire) were weighed, treated for parasites, given vitamins and ear tagged before starting experiment. The ewes were divided into four groups, two control and two treated groups. Each control group had 8 ewes and there were 12 ewes in the treated groups. The rest of the animals were used as replacements, five were "control replacements" and six were treated replacements.

Diets was corn, cobmeal, ground alfalfa hay and 1.4% propionic acid (as preservative). Treated food has "approximately" 100 ppm of TH6040. Rams were rotated with ewes, matting treated rams with treated ewes and control rams with control ewes. Blood samples were taken for analysis. Liver, muscle, fat, spleen and kidney were taken from some experimental sheep. Fetuses and uteri of pregnant ewes were frozen and other simples were placed in dry ice for analysis at Thompson - Hayward. Three control and six treated sheep were lost due to poor physical condition. It would appear, to date, that none of these deaths were caused by "intoxication as evidenced by post mortems.

Results: From the information available to date, there would not appear to be appreciable differences between control ewes and treated ewes or in the progeny of treated or control ewes.

25 '73

Comments: Tissues were taken for sampling but no data is made available. Blood samples were taken for chemical analysis but no data is available.

Validation: Core - Guidelines - tentative to the termination of experiment.

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Section 20 - Vol. II of V

DU 112307

Thirteen Weeks Oral Toxicity Study

In The Sheep

Submitted - June - 1977

PDR 229/77226

Test Compound: Dimilin 90% w/w

Batch no. FL 11/508141

Diflubenzuron pre - mix 90% No. 508141

Test Specie: Sheep

Number of Animals: 24 Dorset Horn Sheep

12 males, 12 females

Route of Administration: Dietary

Doses: 500; 2,500; 10,000 ppm and controls

Testing Laboratory: Huntingdon Research Center

Huntingdon, England

Sponsor: Philips-Duphar, B.V., Weesp, The Netherlands

27 (83)
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Methodology: Twenty-four sheep, 12 males, 12 females were divided into four groups for study. Group one was controls, group two was administered test material in feed at 500 ppm, group three was administered test material at 2,500 ppm and group four at 10,000 ppm. Body weights were recorded weekly. Plasma testosterone and Oestradrol were assayed at 13 weeks. Laboratory investigations and eye examinations were don at 0,4,8 and 13 weeks. Post-mortem examination included organ weight determinations, macroscopic examination and histophathology.

Results: There were no signs of toxicity or abnormal behavior. Animals consumed all the diet offered. Bodyweight gain was within normal limits. Ophthalmological examinations revealed no treatment related abnormalities.

Hematology: Hematological results showed statistical significance at weeks in a decreased hemoglobin at 500 ppm and 2500 ppm with diminished red blood all counts at 500, 2500 and 10,000 ppm. At this time period there was decreased packed cell volume at 2,500 ppm and dimished mean corpuscular volume was also statistically evident at 10,000 ppm. At 8 weeks, mean corpuscular hemoglobin concentration was dimished at the 2500 and 10,000 ppm dose levels.

At 13 weeks, the 2,500 ppm level shows a decreased packed cell volume and hemoglobin content and at 10,000 ppm a decreased mean corpuscular volume. The report states that these statistically

significant values showed no clear pattern or a dose - related response.

Methemoglobinemia: Already at 4 weeks, the first period taken for blood studies, methemoglobinemia values showed increasing values in all treated groups over controls. At 8 weeks, treated groups showed increased values becoming statistively significant at the 2,500 ppm dose level and at 10,000 ppm dose level. At 13 weeks only the 2500 ppm dose level and 10,000 ppm dose level were increased over controls but not significantly.

OD_2 Blood Values: An additional blood dyscrasia was noted appearing with methemoglobinemia. This blood dyscrasia is statistically real and dose related throughout the study appearing at the first period fo blood examinations at 4 weeks (reflected by OD_2 value). There is an increasing trend with dose at the first time period of bolld study-4 weeks at the 500 ppm level with statistical significance at the 2,500 ppm dose level and the 10,000 ppm dose level. The increasing trend with dose is again seen at 8 weeks becoming statistically significant at 10,000 ppm dose level. At thirteen weeks, all treated groups are markedly and statistically increased over controls.

Urine: Specific gravity of urine increased at 8 weeks for both the 500 ppm dose level and the 2,500 ppm level. These increases were

also statistically significant for all treated groups at the 13 weeks period.

Testosterone: Mean plasma testosterone levels at 13 weeks period were 12.13 ng/ml in controls rising to 2.27 ng/ml at the 500 ppm dose level to 5.67 ng/ml at the 2,500 ppm dose with a drop below control values to 1.63 ng/ml at the 10,000 dose level.

Organ Weights: There would appear to be a dimination in thyroid weight in treated groups as compared to control values.

Comments: The obvious blood dyscrasia (measured by optical density) but unidentified as to type needs to be identified. The optical density technique for measurement may not be as sensitive as needed to determine the smallest dose level of DU 112307 that may be ingested that will produce a no-effect level either for methemoglobinemia or other hemoglobinopathy (OD_2 values).

Conclusion: DU 112307 appears to produce methemoglobinemia and other(s) unidentified hemoglobinopathy when fed to sheep at 500; 2,500; and 10,000 ppm in feed for a period of 13 weeks. Other effects observed were decreased thyroid weights, increased and unexplained specific gravity of urine and a normal effect of increasing testostrone levels with dose with a makred decrease of testosterone at the highest dose level.

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Validation: Core - guidelines

Comment: No statement has been submitted regarding duration of treatment.

Validation: Core - Guidelines _ Tentative to submitted of duration of study

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Section 21 Vol 11 of V

Additional Addendum Studies Submitted June 1977

Red Blood Cell and Plasma Cholinesterase Value Following Six Weeks Inclusion of DU 112307 In The Diet of Sheep

Report No.: PDR 229A/77225

Test Compound: DU 112307 - Dimilin
Pre Mix 90% w/w

Test Specie: Sheep

Number of Animals: Twenty-four sheep -
12 males, 12 females
3 males, 3 females/dose level

Route of Administration: Diet

Doses: 0, 500, 2,500, 10,000 ppm

Duration: Six weeks

Laboratory: Huntingdon Research Center
Huntingdon, England

Methodology: Sheep were divided up into four groups, 3 males and 3 females per dose level. Doses were 0, 500, 2,500, 10,000 ppm for a period of six weeks. Cholinesterase values were determined in R.B.C. and plasma for each animal.

Results: Plasma cholinesterase did not differ substantially between groups. R.B.C. cholinesterase did not differ as much between groups as between animals in each group. The variations that exist cannot be attributed to the administration of test material.

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Comment: An experiment of this nature is best designed when each animal acts as its own control also.

Validation: Core - Guidelines

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Additional Data Applicable to PP Nos. 6F1773, 6F1832 and 7F1898

Toxicology Data

Section 22 Vol III of V Addendum Studies

R# 962

June 10, 1977

Effects of DU 112307 (Technical) After Dietary Administration To Male Hubbard Broiler Chickens for 14 Weeks

Test Compound: 1. DU 112307-Technical (Batch No. 405093) purity 99.6%

2. DU 112307-Technical (Batch No. 2866) purity 98.5%

Test Specie: Male Hubbard Broiler Chickens

Number of Animals: 1,700 day old male chickens

Route of Administration: In diet

Doses: 0, 2.5, and 250 ppm

Test Laboratory: Philips Duphar, B.V.
Weesp, The Netherlands

Methodology: One thousand seven hundred male chickens were divided into four groups, two control groups and two treated groups. The basic diet in this study was not constant throughout the study. During the first 56 days the vitamin/mineral mix 188MK was used and from 57 days to end of the test the vitamin/mineral mix 158/MK/158 was used. Mortality, body-weight and food consumption, testosterone and oestradiol values, organ weights, tibia weight and length, comb and

wattle development, gross pathology, microscopic examination and the evaluation of the pictures of the total birds, head and skinned legs were noted.

Results: Not any of the above perameters appeared exceptionally different from controls. However, because of equivocal results from other studies with chickens in regards to testosterone levels, it will be mentioned that the mean test weight at the low level of DU 112307 or at 2.5 ppm was significantly lower $p>0.05$ than controls at 4 weeks. At this period, mean testes weight at the high dose level was also lower than controls but not statistically significant. Mean testosterone levels were lower than controls in the high dose group also at 4 weeks.

Statistically ((T test) there would appear to be $p > .001$) a significant statistical difference between low dose and control dose C_1 and again a statistical difference between high dose and control dose C_2 but there is also a statistical difference between the two control groups C_1 and C_2.

Comment: Due to the large variations in the control values, it is difficult to ascertain that DU 112307 has some effect or no effect upon testosterone level or testes weight in growing chickens in this experiment.

Validation: Core - Supplementary data

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Section 23 Vol V of V

Additional Addendum Studies Submitted June 1977

Isolation and Characterization of Proteoglycans from Chick Limb Bud Chondrocytes Grown in Vitro

Purpose: To determine whether Dimilin significantly intervenes in the incorporation of precursors into preteoglycans.

Test Compound: Dimilin desolved in DMSO/Control DMSO

Test Material: Chick limb bud mesenchymal cells - grown in culture medium facilitating chondrogenic development

Testing Laboratory: Dental Research Institute
Departments of Oral Biology and Biological Chemistry
University of Michigan
Ann Arbor, Michigan 48104

Methodology: The biochemical methodology used is that extensively described in

* "Isolation and Characterization of Proteoglycans from Chick limb Bud. Chondrocytes grown in Vitro by Hascull, Oegema and Brown."

Results: The initial conclusion in the report states "The compound, when administered to the cultures as described above, does not appear to alter significantly the ability of the cells to incorporate 35S into chondriotin sulfate chains and 3H-serine into apparently normal cartilage proteoglycans over a 5 hr. incubation time --."

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* The Journal of Biological Chemistry, Vol 251, No. II.

Section 24 Vol V of V

Additional Addendum Studies Submitted June 1977

Biochemical Effects of Diflubenzuron On Mouse Embryos

Purpose: To determine:

1. Effect of Diflubenzuron (Dimilin) on Mucopolysaccharide synthesis in mouse limb cartilage.
2. Transfer of diflubenzuron across mouse embryonic membranes.
3. Uptake of Diflubenzuron by suckling mice and teratogenic effects.

Test Compound: Diflubenzuron (Dimilin) 99.6% purity

Test Specie: White Swiss mice

Number of Animals: Forty-six mice

Route of Administration: Dietary

Dose: 50 ppm Diflubenzuron

Test Laboratory: Department of Zoology
Brigham Young University
Provo, Utah 84602

Methodology: Female mice were mated with male mice until vaginal plug was observed. Each pregnant female mouse was individually housed and fed Diflubenzuron and ^{14}C Diflubenzuron in feed at 50 ppm. At 17 days, some pregnant females were sacrificed and embryos prepared for residue analysis. The remaining females were permitted to give birth. These lactating females were kept on treatment and permitted to

suckle their young for 13 days at which time the embryos were sacrificed for residue analysis.

Results:

1. Mucopolysaccharide synthesis in embryonic cartilages was normal.
2. Diflubenzuron did not pass through mouse embryonic membranes.
3. Suckling mice did not pick up Diflubenzuron from treated lactating females.
4. Diflubenzuron does not cause teratogenesis in developing mice.